

5 July 2004

Mr. Seth Willey
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RE: Review of Ramey et al. "Testing the taxonomic validity of Preble's Meadow Jumping Mouse (*Zapus hudsonius* (*Z.h.*) *preblei*)"

Dear Seth;

I was recently contacted by your office (in a letter dated 3 June 2004) to review the above report by Dr. Rob Ramey II and co-authors, and herein include my review of that report (also enclosed). It might be helpful to you to know a bit about my background, so that my comments can be judged in the context of my strengths and limitations. As requested, I have also included an updated CV.

First, I am not a mammalogist, and I have not tracked down the taxon-oriented papers cited by Ramey et al. (such as papers or reports by Hafner, Riggs, etc.); I have accepted many things about the biology of these mice (such as the correct identification of taxa sampled for molecular data; see 'Results' section, bottom of p. 7) at face value. I am a herpetologist and my research group focuses almost exclusively on addressing evolutionary and conservation issues in amphibians and reptiles. I have had over 25 yrs of research experience in the use of genetic and molecular markers (allozymes, chromosomes, microsatellites, and sequences of mitochondrial and nuclear gene regions) for population genetic, phylogeographic, and phylogenetic studies of various groups. More to the point of this review, a lot of our work in the past 5-6 yrs has increasingly focused on conservation issues with species of special concern (frogs, salamanders, and turtles in particular), specifically on the issue of treating formally recognized taxonomic entities as hypotheses that should be tested by clearly defined criteria.

I did go through all of the background material sent to me ("Preble's Meadow Jumping Mouse Recovery Plan, Colorado", maps, figures, "Final Rule to List the Preble's Jumping Mouse", etc.) to try to become familiar with the history of the listing of *Z.h. preblei*, the conservation issues, and what has been invested to date in the recovery efforts. I will address each of the points below raised in the initial letter.

1 – Do the morphology, ecology, and mtDNA data presented in the report support the authors' conclusion to synonymize *Z.h. campestris* and *Z.h. preblei*?

The general answer to this question is “yes” but with qualifications. The authors were very clear on p. 4 about setting forth their hypotheses “*a priori*” for testing the taxonomic uniqueness of *Z.h. preblei*, and I am an advocate of this approach (Sites and Crandall, 1997). Ramey et al. adopt the mtDNA “reciprocal monophyly” test of Moritz (1994), and while this test specifies an unambiguous criterion for acceptance/rejection of a distinct ESU (evolutionary significant unit), it does assume that the mtDNA sequences used are evolving under a neutral model (Funk and Omland, 2003). Further, more recent theoretical work suggests that the coalescence of any single genetic locus may have a large variance, even in lineages of the same age, due to many stochastic processes (Irwin, 2002; Hudson and Turelli, 2003).

The mtDNA gene tree presented by Ramey et al. (Fig. 2) is consistent with an interpretation of the introgression *campestris* mtDNA into *Z.h. preblei*, but the topology is asymmetrical and provides no evidence for the reciprocal flow of *preblei* mtDNA back into *Z.h. campestris* – if mating is random in regions of overlap and hybrid and backcross genotypes have approximately the same fitness as parentals, then gene flow should be somewhat symmetrical then we should see at least some *preblei* mtDNA haplotypes introgressing into the Wyoming populations of *campestris*. This pattern would also be expected if hybrid and backcross genotypes showed some fitness reduction relative to parentals, so long as this was approximately the same level of reduction in both directions.

I point out the above alternatives because the gene tree pattern shown in Fig. 2 COULD also result either from: (1) incomplete sorting of mtDNA haplotypes (due to a very recent split between populations); or (2) a “selective sweep” favoring introgression (either ongoing or historically) of *campestris* mtDNA on the *preblei* nuclear background. If incomplete lineage sorting is an issue, then *a priori* one should also see some *preblei* haplotypes in Wyoming populations of *campestris*, because the process is normally stochastic. This appears to be the pattern, for example, between *campestris* and *pallidus* haplotypes: each of these entities segregates a few haplotypes in the “other’s clade” (Fig. 2). The issue of a selective sweep (or the alternative of selection acting against *preblei* mtDNA on the *campestris* nuclear background) is harder to get at, and although I think Ramey et al. make a good case for ongoing gene flow (or at least very recent gene flow) on the basis of absence of morphological differentiation between *campestris* and *preblei*, they did not implement any test of the selective neutrality on their mtDNA sequences (Funk and Omland, 2003; Ballard et al. 2004). They thus cannot unequivocally rule out the possible role of natural selection for the observed phylogeographic patterns of the mtDNA locus (Fig. 2) – if such a sweep has occurred then the “diversity test” proposed by the authors (bottom of p. 4) will fail, and the conclusions drawn at the bottom of p. 9 are compromised (see Rand, 1996, for specific reference of this issue to conservation biology). Fortunately, several statistical tests for neutrality of sequences can be implemented with widely available software (see Morando et al., 2004, for an example), and I would encourage Ramey et al. to do this before submitting their manuscript to a refereed journal.

The strongest case made by Ramey et al. for conspecificity of *Z.h. campestris* and *Z.h. preblei* is the morphological evidence. Here I think their methods are rigorous and unambiguous, the patterns of morphological variation are in general agreement with the recovered mtDNA genealogy, and I agree with their conclusions. One last point;

Ramey et al. implement the “crosshair classification” of Crandall et al. (2000) as a test for ecological distinctiveness (which may reflect adaptive change that would not be detected by molecular markers; p. 5), and then speak of “failure of evidence to reject hypotheses of genetic and ecological exchangeability between *Z.h. preblei* with *Z.h. campestris*, . . . “ (bottom of p. 9). They have not implemented a statistical test of genetic or ecological exchangeability, and so far as I am aware, the only way to do this currently is by implementing Templeton’s (2001) tests for “cohesion species”. Without a statistical test of ecological exchangeability, I don’t think the authors can say much here.

2 – Could you support synonymizing *Z.h. campestris* and *Z.h. preblei* without additional genetics study (i.e., microsatellite data)? If not, what additional analysis is needed and why?

Because so much rests on this decision, I would interpret the Ramey et al. report as tentative evidence for synonymizing *Z.h. campestris* and *Z.h. preblei*, and recommend two things. First, implement tests for neutrality of the mtDNA sequences, and if the null hypothesis (neutrality) cannot be rejected, then Ramey et al. have a stronger basis from which to infer ongoing or historically very recent gene flow between these entities. Second, Ramey et al. (or someone) should definitely conduct a follow-up study using nuclear markers, ideally using the same samples used by Ramey et al. Microsatellites could certainly be used for this, but unless a gene library has already been developed for a closely related species, and unless the microsatellite loci for the “target species” have been shown to amplify (by PCR) and to be polymorphic within *Z.h. campestris*, *Z.h. preblei*, and all other relevant taxa, this will be an expensive and time-consuming approach. I suggest a more feasible follow-up study could be based on “amplified fragment length polymorphisms” (AFLPs); this approach will provide many more loci (albeit they cannot be scored individually as co-dominant) for less cost, and they have been shown to be very informative when trying to assess relationships between closely related taxa (or populations) on the basis of incomplete information from morphological and mtDNA data (see Sullivan et al. 2004, for a recent example). If such a study revealed that the AFLP data recovered both *Z.h. campestris* and *Z.h. preblei* as a single strongly supported clade, the taxonomic conclusion of Ramey et al. would be confirmed. An alternative result might show that these two entities were recovered as separate clades, which would suggest that the mtDNA alone had introgressed asymmetrically from one distinct entity into another (not an uncommon pattern; Funk and Omland, 2003), and that morphological characters were conserved and did not “track” the splitting event.

3 – What is the importance of potential ecological, behavioral, or physiological differences between *Z.h. campestris* and *Z.h. preblei* in substantiating or refuting synonymy?

The ecological points raised by Crandall et al. (2000) are important and might reflect divergence between *Z.h. campestris* and *Z.h. preblei* in some adaptive characteristics driven by ecological differences, and as pointed out by Ramey et al., these

are not likely to be detected by molecular markers. However, without implementation of a rigorous statistical design, such as Templeton (2001), I don't think anything can be added to clarify the issue at present.

4 – What is the likelihood that *Z.h. preblei* is substantially reproductively isolated from other groups within the *Z. hudsonius* complex, especially from *Z.h. campestris*?

See my response to question 2 above – Ramey et al. have a good provisional case for **absence of isolation** between *Z.h. campestris* and *Z.h. preblei*, but follow-up work is needed.

5 – Would the loss of what is now *Z.h. preblei* represent a substantial diminution of the *Z.h. campestris* taxon? Its range, biological characteristics, evolutionary legacy, etc.??

Regardless of the taxonomic reality of *Z.h. preblei* (and I think FWS must delist *Z.h. preblei* if subsequent study validates Ramey et al.; at all costs we need to avoid investing time and resources in bogus taxa that were the result of faulty or incomplete early studies), the map in my supplemental material (Fig. 2, p. 12) shows that what is now *Z.h. preblei* represents a very large and disjunct portion of *Z.h. campestris*, and its loss would almost certainly represent a substantial loss of the evolutionary legacy of *campestris*. This may be especially true in this case because of the peripheral distribution of *Z.h. preblei* relative to *Z.h. campestris* – these kinds of populations are thought to represent a major component of future “speciation potential” of any taxon (Frey, 1993; Lesica and Allendorf, 1995). The application of high resolution molecular markers such as microsatellites might very well reveal that unique alleles are segregating in these peripheral demes, and while such a result would not support continued taxonomic recognition of this entity, it would highlight the evolutionary value of the populations in this part of the range.

In closing, I want to offer my support to the authors for their clear formulation of alternative hypotheses, and criteria by which these were tested in this study. This kind of approach is still frustratingly uncommon, and the authors have done a very good job. I hope my comments here are taken in the context of constructive criticism, and that they will be useful to the authors and to your office.

Sincerely,

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